N1205-003

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
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HERBERT M. WILSON, et al.	)	
	)	
Serial No. 09/140,886	)	Art Unit: 1638
	)	
Filed: August 26, 1998	)	Examiner: O. Zaghmout
	)	
For: **TRANSGENIC PLANTS**	)	

DECLARATION UNDER 37 C.F.R. §1.132

The Honorable Commissioner
of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Herbert Martin Wilson, of 1915 Stevenson Drive, Ames, Iowa 50010, hereby declare that:

I graduated from University of Leicester (United Kingdom) in 1975 with a Ph.D. in Plant Cell Biology.

I was employed by Pfizer, Inc., from 1982 to 1986, where I was a senior scientist in the Plant Genetics Department.

I was employed by ICI Seeds, Inc., from 1986 through 1994 where I was Cell Biology Project Leader.

Since 1995, I have been employed by Stine Seed Company as Director of Stine Biotechnology.

I am one of the co-inventors of the invention described and claimed in the above-identified application and am familiar with the Office Action dated May 18, 2000, in which the Examiner alleges that the disclosure is not enabling.

In order to demonstrate that the disclosure is enabling for claims to transformation of plants with genomic DNA fragments of a donor organism, the experiment set forth below was conducted by me and others under my supervision.

Figure 1 (attached) demonstrates stable expression of the gus gene in five out of six transgenic clones selected on bialaphos (for expression of the bar gene).The unsequenced Sorghum fragment is located between the gus and bar genes (see Fig. 3). Clones (numbered 796-801) were produced by *Agrobacterium*-mediated introduction of the plasmid carrying the unsequenced Sorghum fragment.

Transgenic plant (Fig. 2B) carries the bar gene introduced on the plasmid harboring the unsequenced sorghum DNA fragment. A leaf of this plant was painted with bialaphos and showed no sign of herbicide damage. A nontransformed plant (Fig. 2A) was included as a control. Leaf painting of the control plant with bialaphos produced damage typical of the effect of the herbicide. Figs. 2A and 2B (attached) demonstrate expression of the bar gene providing resistance to bialaphos.

Figure 3 (attached) is a general map of the plasmid used to transform corn. Depicted is the S4 unsequenced-sorghum DNA fragment situated between the gus and bar expression cassettes, all of which are between the right and left T-DNA borders. There are internal Hind III sites in the S4 fragment but they were not shown because they were not mapped.

Figure 4 (attached) demonstrates Southern blot analysis of corn DNA with (lanes 13 and 14) and without (lanes 1-12) the unsequenced-Sorghum S4 DNA fragment. The cloned S4

fragment used to transform the corn (see FIG. 3) resulted from a partial digestion of total Sorghum DNA with Hind III, thus, there were internal Hind III sites within the 9.0 kb S4 fragment. The probe used to generate Southern blot (FIG. 4) was a 4.5 kb Hind III generated, sub-fragment of the 9.0 kb S4 insert. The DNA markers were a 1 kb ladder from Gibco BRL and 1-5 kb locations are noted on right side of the blot. Corn DNAs in lanes 1-14 (about ten micrograms per lane) were digested with Hind III restriction enzyme, run out on a standard agarose gel, blotted, and probed. Lanes 1-12 contain corn DNA that were not transformed with the Sorghum S4 fragment. Lanes 13 and 14 contain corn DNA that were transformed with the unsequenced-Sorghum S4 fragment. There was much cross hybridization detected between the Sorghum and corn DNA. However, in lanes 13 and 14 there is the expected 4.5 kb band that is not clearly detected in the other lanes (see arrows on blot).

Conclusion

The data in this declaration shows that corn plants of the Stine Elite Inbred Line 963 have been stably transformed with a construct that includes the unsequenced Sorghum S4 fragment. The presence of the unsequenced Sorghum S4 fragment is demonstrated in these plants through Southern blot analysis.

I, the undersigned, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United

States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 2/19/01

Herbert Martin Wilson, Ph.D.